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(54) Title: PANCREATIC ISLET GENES REGULATED BY GLUCOSE

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(57) Abstract

The invention relates to novel polynucleotides, including partial and full length cDNA molecules, full-length messenger RNA comprising coding sequence related to the polynucleotides, and polypeptides encoded by the polynucleotides, cDNA, messenger RNA, and methods for producing and using the polynucleotides and polypeptides. The polynucleotides disclosed herein are expressed in pancreatic islet cells, and their expression is regulated by exposure to glucose.

(57) Abrégé

L'invention concerne de nouveaux polynucléotides, ainsi que des molécules partielles et complètes d'ADNc et l'ARN messager complet comprenant des séquences codantes pour ces polynucléotides, les polypeptides codés par ces polynucléotides, cet ADNc, et cet ARN messager, et des procédés de production et d'utilisation de ces polynucléotides et polypeptides. Ces polynucléotides sont exprimés dans les cellules des îlots pancréatiques et leur expression est régulée par l'exposition de ces cellules au glucose.

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(54) Title: PANCREATIC ISLET GENES REGULATED BY GLUCOSE

(57) Abstract

The invention relates to novel polynucleotides, including partial and full length cDNA molecules, full-length messenger RNA comprising coding sequence related to the polynucleotides, and polypeptides encoded by the polynucleotides, cDNA, messenger RNA, and methods for producing and using the polynucleotides and polypeptides. The polynucleotides disclosed herein are expressed in pancreatic islet cells, and their expression is regulated by exposure to glucose.

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Description

PANCREATIC ISLET GENES REGULATED BY GLUCOSE

FIELD OF THE INVENTION

The invention relates to novel polynucleotides, including partial and full-length cDNA molecules, full length messenger RNA comprising coding sequence related to the polynucleotides, full length genes encoding the messenger RNA, and polypeptides encoded by the polynucleotides, and methods for producing and using the polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

The islets of Langerhans are small groups of cells in the pancreas that function as an endocrine gland to secrete the hormones insulin and glucagon. These hormones regulate the sugar content of the blood. Insulin serves to promote the uptake of glucose by cells of the body. Inadequate secretion or utilization of insulin can result in diabetes. The identification of genes whose expression is regulated by glucose levels, particularly genes that are expressed in pancreatic islet cells, provides tools for the understanding, diagnosis and treatment of diabetes and other conditions.

SUMMARY OF THE INVENTION

The invention provides polynucleotides that are differentially expressed in response to glucose. In one embodiment, the invention provides a polynucleotide comprising the nucleotide sequence of any one of SEQ ID NOS:1-17, a polynucleotide that encodes a variant of the polypeptide encoded by the nucleotide sequence, or a polynucleotide that encodes a protein expressed by a polynucleotide having the nucleotide sequence of any one of SEQ ID NOS:1-17. In one embodiment, the polynucleotide is a vector. The invention also provides a host cell transfected with the vector, and a method of producing a polypeptide comprising culturing the host cell and recovering the polypeptide so produced. The invention additionally provides a polypeptide encoded by the nucleotide sequence of any one of SEQ ID NOS:1-17, a

polypeptide encoded by a gene comprising the nucleotide sequence of any one of SEQ ID NOS:1-17 or a variant of one of the above polypeptides.

In addition, the invention provides methods of determining whether a subject exhibits normal expression of a gene regulated by glucose. One such method comprises obtaining a tissue sample from the subject, measuring an expression product of a gene comprising a nucleotide sequence of any one of SEQ ID NOS:1-17 in the sample, and comparing the expression of the measured gene product with expression of the same gene product by normal cells. A difference in expression between the sample and the normal cells is indicative of abnormal expression of a gene regulated by glucose. In one embodiment, the method further comprises contacting the tissue with more than about 5 mM glucose, preferably about 10 to about 30 mM glucose, prior to measuring the expression product of the gene. In one embodiment, the expression product of the gene is measured before and after contacting tissue with glucose.

The invention also provides a method for detecting a human gene comprising one or more of SEQ ID NO:1-17, the method comprising obtaining in computer-readable format at least one of SEQ ID NO:1-17, comparing the sequence with polynucleotide sequences of a human genome, and identifying one or more human genome sequences having at least 95% sequence identity to SEQ ID NO:1-17 as determined by the Smith-Waterman algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1 as parameters.

DETAILED DESCRIPTION OF THE INVENTION

Altered expression of 17 novel genes has been detected in INS-1 cells in response to glucose exposure. These genes are likely to be among those that regulate or manifest the physiological changes brought about by exposure to glucose. The B cells of the pancreatic islet store insulin in secretory granules. Upon an increased blood concentration of, for example, glucose, the cells release the insulin by exocytosis.

INS-1 cells are a highly differentiated rat B cell line and are a suitable model for the cellular changes that occur *in vivo*. See, for example, Kennedy et al.,

1996, *J. Clin. Invest.* *98*:2524-2538, who report on changes in mitochondrial and cytosolic Ca^{2+} levels in INS-1 cells. Thus, this cell line is an appropriate choice for investigating changes in gene expression in response to glucose exposure.

Accordingly, the invention relates to polynucleotides comprising the disclosed nucleotide sequences, to full-length cDNA, mRNA and genes corresponding to these sequences, and to polypeptides and proteins encoded by these polynucleotides and genes. The polynucleotides correspond to genes expressed in pancreatic islet cells, and whose expression is regulated by glucose treatment. The term "nucleic acid" or "polynucleotide" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally-occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence includes the complementary sequence.

Also included are polynucleotides that encode polypeptides encoded by the polynucleotides of the invention. The various polynucleotides that can encode these polypeptides differ because of the degeneracy of the genetic code, in that most amino acids are encoded by more than one triplet codon. The identity of such codons is well-known in this art, and this information can be used for the construction of the polynucleotides within the scope of the invention.

Polynucleotides encoding polypeptides and proteins that are variants of the polypeptides and proteins encoded by the polynucleotides and related cDNA and genes are also within the scope of the invention. The variants differ from wild type protein in having one or more amino acid substitutions, insertions or deletions that enhance, add, or diminish a biological activity of the wild type protein. Once the amino acid change is selected, a polynucleotide encoding that variant is constructed according to the invention.

A polynucleotide will fall within the scope of the invention if it has more than 65% sequence identify, preferably 75% sequence identity, more preferably 85%

sequence identity, and most preferably at least 95% sequence identity, such as 96%, 97%, 98% or 99%, to one of SEQ ID NO:1-17.

The invention also relates to methods of producing and using the disclosed polynucleotide and polypeptide molecules. The molecules can be used to identify functions of the glucose-regulated genes, and in the diagnosis and treatment of diseases such as diabetes.

The invention provides polynucleotides that are differentially expressed in response to glucose treatment. In one embodiment, the invention provides a polynucleotide comprising the nucleotide sequence of any one of SEQ ID NOS:1-17, a polynucleotide that encodes a variant of the polypeptide encoded by the nucleotide sequence, or a polynucleotide that encodes a protein expressed by a polynucleotide having the nucleotide sequence of any one of SEQ ID NOS:1-17. In one embodiment, the polynucleotide is a vector. The invention also provides a host cell transfected with the vector, and a method of producing a polypeptide comprising culturing the host cell and recovering the polypeptide so produced. In addition, the invention provides polynucleotides comprising at least about 12 contiguous nucleotides of any one of SEQ ID NOS:1-17, which can be used, for example, as probes.

The invention also provides polynucleotides having at least 65% sequence identify, preferably at least 75% sequence identity, more preferably at least 85% sequence identity, and most preferably at least 95% sequence identity, such as 96%, 97%, 98% or 99%, to one of SEQ ID NO:1-17, as determined by methods known in the art. A preferred and non-limiting method is the Smith-Waterman homology search algorithm as implemented by the MPSRCH program (Oxford Molecular) using an affine gap search with the following search parameters: gap open penalty of 12, and gap extension penalty of 1.

Thus, the invention encompasses nucleic acid molecules ranging in length from at least about 12 nucleotides (corresponding to at least 12 contiguous nucleotides of one of SEQ ID NOS: 1-17) up to a maximum length suitable for one or more biological manipulations, including replication and expression, of the nucleic acid

molecule. The invention includes, but is not limited to, (a) nucleic acid having the size of a full gene, and comprising at least one of SEQ ID NOS: 1-17; (b) the nucleic acid of all or a portion of (a) also comprising at least one additional gene, operably linked to permit expression of a fusion protein; (c) an expression vector comprising (a) or (b); (d) a plasmid comprising (a) or (b); and (e) a recombinant viral particle comprising (a) or (b). Construction of (a) can be accomplished as described below.

The sequence of a nucleic acid comprising at least 12 contiguous nucleotides of any one of SEQ ID NOS:1-17, preferably the entire sequence of at least any of SEQ ID NOS:1-17, is not limited and can be any sequence of A, T, G, and/or C (for DNA) and A, U, G, and/or C (for RNA) or modified bases thereof, including inosine and pseudouridine. The choice of sequence will depend on the desired function and can be dictated by coding regions desired, the intron-like regions desired, and the regulatory regions desired. Where a sequence of any one of SEQ ID NOS:1-17 is within the nucleic acid, the nucleic acid obtained is referred to herein as a polynucleotide comprising the sequence of any one of SEQ ID NOS: 1-17.

The invention further provides fragments of the disclosed polynucleotide sequences. Preferred fragments are 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 400, 450, 500, 550, 600, 625, 650, 675 or 680 contiguous nucleotides of SEQ ID NO:1; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 365 or 360 contiguous nucleotides of SEQ ID NO:2; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 380, 390, or 400 contiguous nucleotides of SEQ ID NO:3; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 355, 360 or 364 contiguous nucleotides of SEQ ID NO:4; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 305, 310, or 315 contiguous nucleotides of SEQ ID NO:5; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 400, 405 or 409 contiguous nucleotides of SEQ ID NO:6; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350,

375, 400, 425, 450, 475, 500, 525, 550, 575, 580, 590, 600, or 605 contiguous nucleotides of SEQ ID NO:7; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 400, 425, 450, 460, 470, 480, 485, or 487 contiguous nucleotides of SEQ ID NO:8; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 400, 425, 450, 460, 465, 470 or 473 contiguous nucleotides of SEQ ID NO:9; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 400, 425, 450, 460, 465, 470 or 472 contiguous nucleotides of SEQ ID NO:10; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 400, 425, 450, 460, 465, 470, 480, 485, or 490 contiguous nucleotides of SEQ ID NO:11; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 710, 715, 720, 725 or 726 contiguous nucleotides of SEQ ID NO:12; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 710, 715, 720, 725, 730, 740, 750, 760, 765, 770 or 771 contiguous nucleotides of SEQ ID NO:13; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 580, 590, 600, 605, or 607 contiguous nucleotides of SEQ ID NO:14; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 350, 400, 450, 500, 550, 600, 625, 650, 660, 665, 670, or 674 contiguous nucleotides of SEQ ID NO:15; 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 270, 275, 280, 283 or 285 contiguous nucleotides of SEQ ID NO:16; or 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 310, 320, 330, 340, 345, 350, 352, or 353 contiguous nucleotides of SEQ ID NO:17.

The polynucleotides disclosed herein include trans-cleaving catalytic RNAs (ribozymes), which are RNA molecules possessing endoribonuclease activity. Ribozymes are specifically designed for a particular target, and the target message must contain a specific nucleotide sequence. They are engineered to cleave any RNA species

site-specifically in the background of cellular RNA. The cleavage event renders the mRNA unstable and inhibits protein expression. Importantly, ribozymes can be used to inhibit expression of a gene.

The invention provides ribozymes that specifically cleave a polynucleotide comprising the nucleotide sequence of any one of SEQ ID NOS:1-17. In one embodiment, a ribozyme is used to inhibit expression of a gene for the purpose of detecting its function in an *in vitro* or *in vivo* context, by measuring the phenotypic effect. In another embodiment, a ribozyme is used to inhibit expression of a gene to achieve a therapeutic effect.

One commonly used ribozyme motif is the hammerhead, for which the substrate sequence requirements are minimal. Design of the hammerhead ribozyme is disclosed in Usman et al., 1996, *Current Opin. Struct. Biol. 6*:527-533. Usman et al. also discuss therapeutic uses of ribozymes. Ribozymes can also be prepared and used as described in Long et al., 1993, *FASEB J. 7*:25; Symons, 1992, *Ann. Rev. Biochem. 61*:641; Perrotta et al., 1992, *Biochem. 31*:16-17; Ojwang et al., 1992, *Proc. Natl. Acad. Sci.* (USA) *89*:10802-10806; U.S. Patent No. 5,254,678. Ribozyme cleavage of HIV-I RNA is described in U.S. Patent No. 5,144,019; methods of cleaving RNA using ribozymes are described in U.S. Patent No. 5,116,742; and methods for increasing the specificity of ribozymes are described in U.S. Patent No. 5,225,337 and Koizumi et al., 1989, *Nucleic Acid Res. 17*:7059-7071. Preparation and use of ribozyme fragments in a hammerhead structure are also described by Koizumi et al., 1989, *Nucleic Acid Res. 17*:7059-7071. Preparation and use of ribozyme fragments in a hairpin structure are described by Chowrira and Burke, 1992, *Nucleic Acids Res. 20*:2835. Ribozymes can also be made by rolling transcription as described in Daubendiek and Kool, 1997, *Nat. Biotechnol. 15*(3)273-277.

The invention provides antisense nucleic acids that specifically bind to polynucleotides comprising the nucleotide sequence of any one of SEQ ID NOS:1-17. Antisense nucleic acids are designed to specifically bind to RNA, resulting in the formation of RNA-DNA or RNA-RNA hybrids, and inhibition of DNA replication,

reverse transcription or messenger RNA translation. Antisense polynucleotides based on a selected cDNA sequence can interfere with expression of the corresponding gene. Antisense polynucleotides are typically generated within the cell by antisense constructs that express polynucleotides complimentary to the mRNA. Antisense cDNA-encoded polynucleotides will bind and/or interfere with the translation of cDNA-related mRNA. The expression products of control cells and cells treated with the antisense construct are compared to detect the level of protein product of the gene corresponding to the cDNA. The protein is isolated and identified using routine biochemical methods.

The promoter region of a gene generally is located 5' to the initiation site for RNA polymerase II. Hundreds of promoter regions contain the "TATA" box, a sequence such as TATTA or TATAA, which is sensitive to mutations. The promoter region can be obtained by performing 5' RACE using a primer from the coding region of the gene. Alternatively, the cDNA can be used as a probe for the genomic sequence, and the region 5' to the coding region is identified by "walking up."

Because the disclosed polynucleotides are differentially expressed, the promoters from the corresponding genes can be used in a regulatory construct for a heterologous gene. For example, the 5' regulatory regions corresponding to SEQ ID NOS:1-15, genes whose expression is up-regulated in response to glucose treatment, can be used to achieve glucose-inducible expression of a heterologous gene. For applications in which it is desirable to achieve reduced expression under high glucose conditions, the 5' regulatory regions corresponding to SEQ ID NOS:16-17 can be used to regulate expression of a heterologous gene.

A human gene comprising at least one of SEQ ID NO:1-17 can be identified and isolated using methods known in the art. According to one method, one or more of SEQ ID NO:1-17 is prepared in a computer-readable format. The sequence is compared with polynucleotide sequences of a human genome, and one or more human genome sequences having at least 95% sequence identity to one or more of SEQ ID NO:1-17 are identified, for example by using the Smith-Waterman algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1 as

parameters. Probes based on the regions of homology between SEQ ID NO:1-17 and the human genome sequences are prepared and used to isolate polynucleotides from human genomic DNA, using methods known in the art. The invention includes human genomic DNA comprising the coding region of any one of SEQ ID NO:1-17 and any untranslated regions which do not share homology with SEQ ID NO:1-17 but which are contiguous with homologous regions. Such genomic DNA includes but is not limited to introns, promoters, and other regulatory regions functionally associated with a human gene having a region corresponding to SEQ ID NO:1-17.

The cDNA of any one of SEQ ID NOS:1-17 itself, the corresponding full-length cDNA, or the full-length gene is used to express the partial or complete gene product. Appropriate polynucleotide constructs are purified using standard recombinant DNA techniques as described in, for example, Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Press, Cold Spring Harbor, New York), and under current regulations described in United States Dept. of HHS, National Institute of Health (NIH) Guidelines for Recombinant DNA Research. The polypeptides encoded by the cDNA are expressed in any expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems. Suitable vectors and host cells are described in U.S. Patent No. 5,654,173.

Polynucleotide molecules of the invention can be propagated by placing the molecule in a vector. As used herein, a vector is a construct which is capable of delivering, and preferably expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

Viral and non-viral vectors are used, including plasmids. The choice of plasmid will depend on the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for transfer and expression in

cells in a whole animal or person. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially. The cDNA or full-length polynucleotide is inserted into a vector typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence may be inserted by homologous recombination *in vivo*. Typically this is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence. Regions of homology are added by ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence, for example.

cDNAs or full-length polynucleotides are linked to regulatory sequences as appropriate to obtain the desired expression properties. These may include promoters (attached either at the 5' end of the sense strand or at the 3' end of the antisense strand), enhancers, terminators, operators, repressors, and inducers. The promoter may be regulated or constitutive. In some situations it may be desirable to use conditionally active promoters, such as tissue-specific or developmental stage-specific promoters. These are linked to the desired nucleotide sequence using the techniques described above for linkage to vectors. Any techniques known in the art may be used.

When any of the above host cells, or other appropriate host cells or organisms, are used to replicate and/or express the polynucleotides or nucleic acids of the invention, the resulting replicated nucleic acid, RNA, expressed protein or polypeptide, is within the scope of the invention as a product of the host cell or organism. The product is recovered by any means known in the art.

Where the methods discussed herein require sequence alignment, such methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison may by conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math. 2*:482; by the homology alignment algorithm of Needleman and Wunch (1970) *J. Mol. Biol. 48*:443; by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA 85*:2444; by computerized implementations of these algorithms (including, but not

limited to CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California, GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wisconsin, USA); the CLUSTAL program as described by Higgins and Sharp (1988) *Gene, 73*:237-244 and Higgins and Sharp (1989) *CABIOS 5*:151-153; Corpet et al., (1988) *Nucleic Acids Research 16*, 10881-90; Huang et al., (1992) *Computer Applications in the Biosciences 8*, 155-65, and Pearson et al., (1994) *Methods in Molecular Biology 24*, 307-31. Typically, the alignments are visually inspected and refined manually after computer-aided adjustment.

The polypeptides of the invention include those encoded by the disclosed polynucleotides. These polypeptides can also be encoded by nucleic acids that, by virtue of the degeneracy of the genetic code, are not identical in sequence to the disclosed cDNAs. Thus, the invention includes within its scope nucleic acids comprising polynucleotides encoding a protein or polypeptide expressed by a polynucleotide having the sequence of any one of SEQ ID NOS:1-17. Also within the scope of the invention are variants; variants of polypeptides include mutants, fragments, and fusions. Mutants can include amino acid substitutions, additions or deletions. The amino acid substitutions can be conservative amino acid substitutions or substitutions to eliminate non-essential amino acids, such as to alter a glycosylation site, a phosphorylation site or an acetylation site, or to minimize misfolding by substitution or deletion of one or more cysteine residues that are not necessary for function. Conservative amino acid substitutions are those that preserve the general charge, hydrophobicity/hydrophilicity, and/or steric bulk of the amino acid substituted. For example, substitutions between the following groups are conservative: Gly/Ala, Val/Ile/Leu, Asp/Glu, Lys/Arg, Asn/Gln, Ser/Cys, Ther, and Phe/Trp/Tyr.

Expression products of a cDNA, the corresponding mRNA, or the corresponding complete gene are prepared and used for raising antibodies for experimental, diagnostic, and therapeutic purposes. The cDNA is expressed as described above, and antibodies are prepared. These antibodies are specific to an

epitope on the cDNA-encoded polypeptide, and can precipitate or bind to the corresponding native protein in a cell or tissue preparation or in a cell-free extract of an *in vitro* expression system.

Immunogens for raising antibodies are prepared by mixing the polypeptides encoded by the cDNAs of the present invention with adjuvants. Alternatively, polypeptides are made as fusion proteins to larger immunogenic proteins. Polypeptides are also covalently linked to other larger immunogenic proteins, such as keyhole limpet hemocyanin. Immunogens are administered to experimental animals such as rabbits, sheep, and mice, to generate antibodies. Optionally, the animal spleen cells are isolated and fused with myeloma cells to form hybridomas that secrete monoclonal antibodies. Such methods are well known in the art. According to another method known in the art, the cDNA-related polynucleotide is administered directly, such as by intramuscular injection, and expressed *in vivo*. The expressed protein generates a variety of protein-specific immune response, including production of antibodies, comparable to administration of the protein.

Preparations of polyclonal and monoclonal antibodies specific for cDNA-encoded proteins and polypeptides are made using standard methods known in the art. The antibodies specifically bind to epitopes present in the polypeptides encoded by polynucleotides disclosed in the Sequence Listing. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, for example at least 15, 25, or 50 amino acids.

Antibodies that specifically bind to human polypeptides should provide a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in Western blots or other immunochemical assays. Preferably, antibodies that specifically bind polypeptides of the invention do not detect other proteins in immunochemical assays and can immunoprecipitate the corresponding proteins from solution.

To test for the presence of serum antibodies to the polypeptides of the invention in a human population, human antibodies are purified by methods well known in the art. Preferably, the antibodies are affinity purified by passing antiserum over a column to which a cDNA-encoded protein, polypeptide, or fusion protein is bound. The bound antibodies can then be eluted from the column, for example using a buffer with a high salt concentration.

In addition to the antibodies discussed above, genetically engineered antibody derivatives are made, such as single chain antibodies. Recombinant antibody libraries can be screened to identify useful molecules.

Polypeptides encoded by the instant cDNAs and corresponding full length genes can be used to screen peptide libraries to identify binding partners, such as receptors, from among the encoded polypeptides. A library of peptides may be synthesized following the methods disclosed in U.S. Pat. No. 5,010,175, and in PCT WO 91/17823.

Abnormal expression of a gene corresponding to any one of SEQ ID NOS:1-17 may be linked to abnormalities or diseases associated with altered sensitivity to glucose levels, such as diabetes, including type 1 or type 2 diabetes. The ability to detect abnormal expression of such genes provides a useful tool for diagnosing diabetes and for developing effective treatment strategies.

The invention provides a method of determining whether a subject exhibits abnormal expression of a gene regulated by glucose. The method comprises obtaining a tissue sample from the subject, measuring an expression product of a gene comprising a nucleotide sequence of any one of SEQ ID NOS:1-17 in the sample, and comparing the expression of the measured gene product with expression of the same gene product by normal cells. A difference in expression between the sample and the normal cells is indicative of abnormal expression of a gene regulated by glucose. In one embodiment, the method further comprises contacting the tissue with more than about 5 mM glucose, preferably about 10 to about 30 mM glucose, prior to measuring the expression product of the gene. In one embodiment, the expression product of the gene

is measured before and after contacting tissue with glucose. Examples of expression products of the gene that can be measured include cDNA, mRNA and protein. These products can be measured using standard techniques, including RT-PCR, northern blotting and western blotting, for example. The foregoing steps can also be used in a method for diagnosing diabetes, or for identifying different patterns of abnormality to facilitate development of an effective treatment strategy, particularly for humans.

Polynucleotide arrays provide a high throughput technique for assaying a large number of polynucleotide sequences in a sample. This technology can be used as a diagnostic and as a tool to test for differential expression to determine function of an encoded protein.

To create arrays, polynucleotide probes are spotted onto a substrate in a two-dimensional matrix or array. Samples of polynucleotides can be labeled and then hybridized to the probes. Double stranded polynucleotides, comprising the labeled sample polynucleotides bound to probe polynucleotides, can be detected once the unbound portion of the sample is washed away.

The probe polynucleotides can be spotted on substrates including glass, nitrocellulose, etc. The probes can be bound to the substrate by either covalent bonds or by non-specific interactions, such as hydrophobic interactions. The sample polynucleotides can be labeled using radioactive labels, fluorophors, etc.

Techniques for constructing arrays and methods of using these arrays are described in EP No. 0 799 897; PCT No. WO 97/29212; PCT No. WO 97/27317; EP No. 0 785 280; PCT No. WO 97/02357; U.S. Pat. No. 5,593,839; U.S. Pat. No. 5,578,832; EP No. 0 728,520; U.S. Pat. No. 5,599,695; EP No. 0 721 016; U.S. Pat. No. 5,556,752; PCT No. WO 95/22058; and U.S. Pat. No. 5,631,734.

The invention provides compositions and methods for treatment of disease, such as diabetes. In one embodiment, the composition is a pharmaceutical composition. Pharmaceutical compositions can comprise polypeptides, antibodies, or polynucleotides of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides

of the invention. As used herein, pharmaceutically acceptable carrier includes any material which, when combined with an active ingredient of a composition, allows the ingredient to retain biological activity and is essentially non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline or normal (0.9%) saline.

Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, Chapter 43, 14th Ed., Mack Publishing Co., Easton PA 18042, USA).

The term therapeutically effective amount as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. The effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers

may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., N.J. 1991). As used herein, pharmaceutically acceptable salt refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects. Examples of such salts include, but are not limited to, (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, polygalacturonic acid; (b) salts with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, and the like; or (c) salts formed with an organic cation formed from N,N'-dibenzylethylenediamine or ethylenediamine; or (d) combinations of (a) and (b) or (c), e.g., a zinc tannate salt; and the like. The preferred acid addition salts are the trifluoroacetate salt and the acetate salt.

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for

solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Once formulated, the polynucleotide compositions of the invention can be (1) administered directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) delivered *in vitro* for expression of recombinant proteins.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., International Publication No. WO 93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoetic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Once a gene has been found to correlate with a disorder, such as diabetes, the disorder may be amenable to treatment by administration of a therapeutic agent based on the cDNA or corresponding polypeptide. In one embodiment, the therapeutic agent comprises an antisense molecule. Therapeutic agents also include antibodies to proteins and polypeptides encoded by the cDNAs and related genes, as described in U.S. Patent No. 5,654,173.

Both the dose of the antisense composition and the means of administration are determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. Administration of the therapeutic antisense agents of the invention includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. Preferably, the therapeutic antisense composition contains an expression construct comprising a promoter and a polynucleotide segment of at least 12, 22, 25, 30, or 35 contiguous nucleotides of the antisense strand of a cDNA. Within the expression construct, the polynucleotide segment is located downstream from the promoter, and transcription of the polynucleotide segment initiates at the promoter. As used herein, an expression control sequence is a nucleic acid sequence which directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence can be operably linked to a nucleic acid sequence to be transcribed.

Receptor-mediated targeted delivery of therapeutic compositions containing an antisense polynucleotide, subgenomic polynucleotides, or antibodies to specific tissues is also used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., 1993, *Trends in Biotechnol. 11*:202-205; Chiou et al., 1994, Gene Therapeutics: Methods and Applications of Direct Gene Transfer (J.A. Wolff, ed.); Wu & Wu, 1988, *J. Biol. Chem. 263*:621-24; Wu et al., 1994, *J. Biol. Chem. 269*:542-46; Zenke et al., 1990, *Proc. Natl. Acad. Sci.* (USA) *87*:3655-59; Wu et al., 1991, *J. Biol. Chem. 266*:338-42. Preferably, receptor-mediated targeted delivery of therapeutic compositions containing antibodies of the invention is used to deliver the antibodies to specific tissue.

Therapeutic compositions containing antisense subgenomic polynucleotides are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20

μg to about 100 μg of DNA can also be used during a gene therapy protocol. Factors such as method of action and efficacy of transformation and expression are considerations which will affect the dosage required for ultimate efficacy of the antisense subgenomic polynucleotides. Where greater expression is desired over a larger area of tissue, larger amounts of antisense subgenomic polynucleotides or the same amounts readministered in a successive protocol of administrations, or several administrations to different adjacent or close tissue portions may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect. A more complete description of gene therapy vectors, especially retroviral vectors, is contained in U.S. Serial No. 08/869,309, which is expressly incorporated herein.

The gene delivery vehicle may be of viral or non-viral origin (see generally, Jolly, 1994, *Cancer Gene Therapy 1*:51-64; Kimura, 1994, *Human Gene Therapy 5*:845-852; Connelly, 1995, *Human Gene Therapy 1*:185-193; and Kaplitt, 1994, *Nature Genetics 6*:148-153). Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches. Expression of such coding sequences can be either constitutive or regulated.

The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

EXAMPLES

Example 1: Identification of Genes Differentially Expressed in Response to Glucose Treatment

Rat pancreatic islet cells of the INS-1 cell line (Kennedy et al., 1996, *J. Clin. Invest. 98*:2524-2538) were treated with Krebs Ringer Bicarbonate buffer (KRBH) containing either 3 mM (low) glucose or 20 mM (high) glucose for 60 minutes. Total

RNA was prepared and analyzed according to the manufacturer's instructions using 200 primer pairs and HIEROGLYPH mRNA profile kit for differential expression (Genomyx, Palo Alto, CA). Differentially expressed bands were cut out and subcloned into a plasmid vector for expression. DNA was prepared and sequenced.

Example 2: Analysis of Differentially Expressed Polynucleotides

Sequences identified in Example 1 were then analyzed using BLAST (available at http://www.ncbi.nlm.nih.gov/BLAST/) and by alignment with publicly available sequences (Genbank, NRP). SEQ ID NOS:1-15 were up-regulated in cells treated with high glucose, while SEQ ID NOS:16-17 were down-regulated in response to high glucose. The sequences are shown in Table 1.

Deposit Information:

The following materials were deposited with the American Type Culture Collection:

Name	Deposit Date	ATCC Accession No.	CMCC Accession No.
Bacterial Cultures	09/28/98	98897	4850

The above material has been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A., under the accession number indicated. The deposit consists of a mixture of 17 bacterial cultures each containing a plasmid having a cDNA insert as described in the application. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for purposes of Patent Procedure. The deposit will be maintained for a period of 30 years following issuance of this patent, or for the enforceable life of the patent, whichever is greater. Upon issuance of the patent, the deposit will be available to the public from the ATCC without restriction.

This deposit is provided merely as convenience to those of skill in the art, and is not an admission that a deposit is required under 35 U.S.C. § 112. The sequence of the polynucleotides contained within the deposited material, as well as the

amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with the written description of sequences herein. A license may be required to make, use, or sell the deposited material, and no such license is granted hereby.

The foregoing detailed description provides exemplary information about the invention. Those skilled in the art will appreciate that modifications can be made without diverging from the spirit and purpose of the invention.

Claims

CLAIMS

What is claimed is:

1. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleic acid sequence of any one of SEQ ID NOS:1-17;

(b) a polynucleotide encoding a polypeptide expressed by a polynucleotide comprising any one of SEQ ID NOS: 1-17;

(c) the polynucleotide complement of the polynucleotide of (a) or (b); and

(d) a polynucleotide at least 90% identical to the polynucleotide of (a), (b), or (c).

2. The polynucleotide of claim 1 comprising the nucleotide sequence of any one of SEQ ID NOS: 1-17.

3. An isolated nucleic acid molecule, which comprises 10-280 contiguous nucleotides of one of SEQ ID NO:1-17, 10-400 contiguous nucleotides of one of SEQ ID NO:1, 3, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15; 10-600 contiguous nucleotides of one of SEQ ID NO:7, 12, 13, 14 and 15; or 10-700 contiguous nucleotides of one of SEQ ID NO:12 and 13.

4. The isolated nucleic acid molecule of claim 3, which comprises 100-280 contiguous nucleotides of one of SEQ ID NO:1-17.

5. The isolated nucleic acid molecule of claim 4, which comprises 150-200 contiguous nucleotides of one of SEQ ID NO:1-17.

6. The isolated nucleic acid molecule of claim 1, which is DNA.

7. A method of making a recombinant vector comprising inserting a nucleic acid molecule of claim 1 into a vector in operable linkage to a promoter.

8. A recombinant vector produced by the method of claim 7.

9. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 8 into a host cell.

10. A recombinant host cell produced by the method of claim 9.

11. A recombinant method of producing a polypeptide, comprising culturing the recombinant host cell of claim 10 under conditions such that said polypeptide is expressed and recovering said polypeptide.

12. A method of determining whether a mammalian subject exhibits abnormal expression of a gene regulated by glucose, comprising:

(a) obtaining a tissue sample from the mammalian subject;

(b) measuring an expression product of a gene comprising a nucleotide sequence of any one of SEQ ID NOS:1-17 in the sample; and

(c) comparing the expression of the gene product measured in step (b) with expression of the same gene product by normal cells, a difference in expression between the sample and the normal cells being indicative of abnormal expression in said tissue sample of a gene regulated by glucose.

13. The method of claim 12, further comprising contacting the tissue of step (a) with more than about 5 mM glucose prior to measuring the expression product of the gene.

14. The method of claim 13, wherein the tissue is contacted with about 10 to about 30 mM glucose.

15. The method of claim 13, wherein the expression product of the gene is measured before and after contacting tissue with more than about 5 mM glucose.

16. The method of claim 12, wherein the expression product of the gene is cDNA, mRNA or protein.

17. A method for detecting a human gene comprising any one of SEQ ID NO:1-17, said method comprising obtaining in computer-readable format said one of SEQ ID NO:1-17, comparing said sequence with polynucleotide sequences of a human genome, and identifying one or more human genome sequences having at least 95% sequence identity to said one of SEQ ID NO:1-17 as determined by the Smith-Waterman algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1 as parameters.

18. A non-naturally occurring fusion protein comprising a first protein segment and a second protein segment fused to each other by means of a peptide bond, wherein the first protein segment comprises at least six contiguous amino acids selected from an amino acid sequence encoded by the nucleotide sequence of any one of SEQ ID NO:1-17 or the complement thereof.

19. The fusion protein of claim 18 wherein said first protein segment comprises at least six contiguous amino acids encoded by said one of SEQ ID NO:1-17.

20. The fusion protein of claim 19 wherein said first protein segment comprises at least twelve contiguous amino acids encoded by said one of SEQ ID NO:1-17.

21. The fusion protein of claim 20 wherein said first protein segment comprises at least 50 contiguous amino acids encoded by said one of SEQ ID NO:1-17.

22. The fusion protein of claim 21 wherein said first protein segment comprises at least 80 contiguous amino acids encoded by said one of SEQ ID NO:1-17.

23. An isolated antibody that binds specifically to a polypeptide encoded by the polynucleotide of claim 1.

SEQUENCE LISTING

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Haidong Xu

<120> NOVEL PANCREATIC ISLET GENES REGULATED BY GLUCOSE

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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

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(60) Parent Application or Grant
CHIRON CORPORATION [/]; (). KENNEDY, Giulia [/]; (). XU, Haidong [/]; (). BLACKBURN, Robert, P. ; ().

Published

(54) Title: PANCREATIC ISLET GENES REGULATED BY GLUCOSE

(54) Titre: GENES DES ILOTS PANCREATIQUES REGULES PAR LE GLUCOSE

(57) Abstract

The invention relates to novel polynucleotides, including partial and full length cDNA molecules, full-length messenger RNA comprising coding sequence related to the polynucleotides, and polypeptides encoded by the polynucleotides, cDNA, messenger RNA, and methods for producing and using the polynucleotides and polypeptides. The polynucleotides disclosed herein are expressed in pancreatic islet cells, and their expression is regulated by exposure to glucose.

(57) Abrégé

L'invention concerne de nouveaux polynucléotides, ainsi que des molécules partielles et complètes d'ADNc et l'ARN messager complet comprenant des séquences codantes pour ces polynucléotides, les polypeptides codés par ces polynucléotides, cet ADNc, et cet ARN messager, et des procédés de production et d'utilisation de ces polynucléotides et polypeptides. Ces polynucléotides sont exprimés dans les cellules des îlots pancréatiques et leur expression est régulée par l'exposition de ces cellules au glucose.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(74) Agents: BLACKBURN, Robert, P.; Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608-2916 et al. (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

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Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/46372 A3

(54) Title: PANCREATIC ISLET GENES REGULATED BY GLUCOSE

(57) Abstract: The invention relates to novel polynucleotides, including partial and full length cDNA molecules, full-length messenger RNA comprising coding sequence related to the polynucleotides, and polypeptides encoded by the polynucleotides, cDNA, messenger RNA, and methods for producing and using the polynucleotides and polypeptides. The polynucleotides disclosed herein are expressed in pancreatic islet cells, and their expression is regulated by exposure to glucose.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/03108

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 C12Q1/68 C12N15/62 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LEIBIGER B ET AL: "Short-term regulation of insulin gene transcription by glucose" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 95, 1998, pages 9307-9312, XP002142532 WASHINGTON US	

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 2000	18 OCT 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ESPEN, J

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/03108

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

invention 1, in part. claims 1-23 all as far as applicable

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: Invention 1; Claims: in part: 1-23; all as far as applicable

Polynucleotide relating to SEQ ID NO 1, and fragments thereof. Vector comprising such a polynucleotide and host cells comprising said vector. Method of producing a polypeptide by culturing said host cell. Method of determining whether a mammalian subject exhibits abnormal expression of a gene regulated by glucose. Method for detecting a human gene. Fusion protein relating to SEQ ID NO 1.

Inventions 2-17; Claims: in part 1-23; all as far as applicable

as invention 1 but limited to subject-matter relating to SEQ ID NOs 2-17; wherein
invention 2 is limited to SEQ ID NO 2
invention 3 is limited to SEQ ID NO 3, etc...
invention 17 is limited to SEQ ID NO 17